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Original Article

Antimicrobial Potential of Flavonoids (Free and Bound) of *Euphorbia hirta* against Selected Dermatophytes Strains

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ABSTRACT

Background: Dermatophytosis/Tinea/ringworm is one of the most infectious diseases of humans, caused by invasion of stratum corneum by dermatophytic fungi viz. *Trichophyton* species, *Microsporum* species and *Epidermophyton* species, though not fatal infections are unpleasant, unhygienic to have and re-occurrence is very common. In recent years, especially during and after COVID 19 pandemic, the development of natural products to produce more cost-effective remedies for different diseases that are affordable to the common man is now being explored from plants. *Euphorbia hirta* of the family *Euporbiaceae* is well known for its medicinal properties in traditional and folklore medicinal systems. *Euphorbia hirta* possesses antifungal properties that may be effective against dermatophytic fungi responsible for causing ringworm. **Materials and Methods:** Different parts of the plant were taken for the extraction of flavonoids by a well-established protocol with some modifications. Agar well diffusion method, Two-fold dilution method and Total activity determination were carried out to establish the antidermatophytic activity of flavonoids from different parts of the plant **Results:** Significant Inhibition Zone (IZ) and Activity Index (AI) were observed against *Microsporum canis, Trichophyton rubrum*, and *Epidermophyton floccosm* from free flavonoid of the leaf; bound flavonoid of fruit; and bound flavonoid of the root. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) recorded were more or less similar to that of standard drugs. **Conclusion:** The present study suggests metabolite profiling of different parts of *E. hirta*. Flavonoids from the plant could be used as a source for the development of alternative drugs for the topical treatment of dermatophytosis.

Keywords: Skin disorders, Dermatophytes, *Euphorbia hirta*, MIC, MFC.

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INTRODUCTION

Skin-related conditions are widespread health problems that harm people of all ages, from children to the elderly and do so in several ways. Numerous native plants and their components are frequently used to treat several medical ailments.[1] The dermatophytes are a group of keratinophilic fungi, that can penetrate, infect and feed upon keratinized tissue (skin, hair and nails) in humans and animals as well, responsible for causing Dermatophytosis, commonly known as Ringworm or Tinea.[2] Dermatophytosis is considered one of the most infectious diseases of humans, though not fatal, or life-threatening but causes discomfort and irritation in patients. It is a common global public health problem, especially in tropical countries such as India where moist climatic conditions facilitate fungal infections.[3] Skin-related diseases including ringworm are often neglected by patients and the health sector as not being life-threatening. This particular field remains insufficiently financed and understaffed at the level of health centers and awareness among patients for maintaining hygiene and cleanliness is lacking throughout rural and urban areas (especially socio-economic deprived areas) of developing countries. It has long been challenging to adequately treat fungal diseases because infection re-occurs with time and is often not cured completely due to the emergence of resistant strains even after following prescribed treatment. Therefore, exploration of new sources of anti-fungal drugs is always welcoming and should be done on a priority basis because fungal infections may worsen and be associated with other microbial infections as well.

Numerous antimicrobial compounds, including those with antidermatophytic properties, have been identified in medicinal plants in significant quantities, many of which are already used in traditional medicine practices. Some plants have been well-known to cure skin diseases since ancient times. Neem (*Azadirachta indica*), Palas (*Butea monosperma*) and turmeric (*Curcuma longa*) are widely recognized for their antibiotic properties. In the pursuit of discovering new antibiotic compounds, plant extracts

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and metabolites are being investigated to uncover their untapped bioactive potential.[4]

In general, plants create an extensive number of secondary metabolites, which make up a significant source of microbicides, insecticides and numerous pharmaceuticals. The primary source of pharmacological compounds utilized in traditional medicine is medicinal plants. This is because substances from nature have a higher level of chemical variability and innovative molecules are not generated from other sources.[5] The rich biodiversity of India makes it a home for an enormous variety of therapeutic medicinal plants. Medicinal plants are a significant component of the traditional healthcare systems of tropical countries because herbal remedies are more secure and sustainable than artificial drugs. The bioactive chemicals found in these medicinal plants have been used to create several medications since ancient times.[6] The usage of phytomedicines and the revival of green medicine have grown significantly in recent years, especially during the COVID-19 pandemic. These natural substances mainly consist of secondary metabolites, such as flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenolic compounds. As a source of phytomedicine, phytonutrients with unidentified pharmacological properties have received a lot of attention recently.[7]

Euphorbia hirta of the family *Euporbiaceae* was selected for the present investigation as it is being used to cure several skin diseases in folklore medicine. Thus, making it a suitable and potential raw source for the exploration of antidermatophytic compounds of herbal origin. It is a little annual herb that is widespread in tropical regions, also known as spurge bears pills and asthma herb. The stem is slender, reddish in colour and coated in yellowish bristly hair. *Euphorbia hirta* has oppositely oriented, roughly 5 cm long leaves that are typically reddish or greenish below.[8]

In the present investigation, flavonoids from different plant parts of *Euphorbia hirta* were evaluated for antidermatophytic activity against three test dermatophytes i.e. *Microsporum canis, Trichophyton rubrum*, and *Epidermophyton floccosum*.

MATERIALS AND METHODS

Collection and Authentication of Plants

Different parts of *Euphorbia hirta* (root, stem, leaf and fruits) were collected from different areas and localities of Jaipur and Tonk, Rajasthan. A voucher specimen was submitted to the Herbarium, Department of Botany, University of Rajasthan, Jaipur, for authentication and the number RUBL 21205 was given to it. The whole plant was thoroughly rinsed with tap water and shade-dried at room temperature. Then plan samples were ground to a fine powder and stored in air-tight boxes for further use (Figures 8 and 9).

Preliminary detection test for flavonoids

Sample plant parts (aqueous extracts and powdered) were subjected to preliminary detection tests for flavonoid plants.[9] The following methods were used to determine the presence of flavonoids.

NaOH Test: The appearance of yellow colour with addition of NaOH to the test sample indicates the presence of flavonoids in the sample.

Ethyle acetate Test: A portion of the powered plant sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 1 mL of dilute ammonia solution was added to 4 mL of filtrate. A yellow coloration was observed, indicating a positive test for flavonoids.

Ammonia Test 5 mL of diluted ammonia solution was added to the aqueous plant extract, followed by the addition of con. H_2SO_4. Yellow colour was observed in each extract, indicating the presence of flavonoids. The Yellow colour disappeared on standing.

Shinoda Test To each test sample a piece of magnesium ribbon and concentrated HCl were added dropwise. A pink, scarlet, crimson, or occasionally green or blue colour indicated the presence of flavonoid.

Extraction of flavonoids

Different plant parts such as the stem, leaf, fruit and root of *Euphorbia hirta* were subjected to flavonoid extraction following the method.[10] 100 g of a finely powdered sample of each plant part was Soxhlet extraction with 80% of methanol in a water bath for 24 hr and filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate. Petroleum ether fractions were discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analysed for free and bound flavonoids. The ethyl acetate fraction of each of the samples was hydrolysed by refluxing with 7 % sulfuric acid for 2 hr. The resulting mixture was filtered and the filtrate was extracted with ethyl acetate which was then washed with distilled water to neutrality ethyl ether (Free Flavonoids) and Ethyle acetate (Bound Flavonoids), was dried, weight and stored at 4°C in air-tight glass vials for future uses. Different concentrations (0.5 mg/mL, 1 mg/mL, 1.5 mg/mL and 2 mg/mL) of the free and bound flavonoids from *Euphorbia hirta* were preparing using Dimethyl Sulfoxide (DMSO).

Test Dermatophytes

Selected dermatophytes (causal organisms of dermatophytosis) were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. Selected dermatophytes were cultured on Sabouraud Dextrose Agar media (SDA) and subculture regularly as recommended by MTCC. All the agar slants and broth were kept at 4°C in the refrigerator for future studies.

Dermatophyte strain MTCC No.

Microsporum canis 2820
Trichophyton rubrum 3272
Epidermophyton floccosum 7880

Agar well diffusion method

'Agar well diffusion assay' was followed for the determination of the antidermatophytic potential of free and bound flavonoids of *Euphorbia hirta* .[11] SDA base plates were seeded with the standard size of fungal inoculum (1× 10^6 CFU/mL for dermatophytic fungi). Twenty µL of inoculum (test dermatophyte) was aseptically introduced by micropipette and spread using a sterile glass spreader on agar plates. A well of 6.0 mm diameter was aseptically punched on each agar plate with a sterile cock borer. The experiment was carried out in triplicate. Free and bound flavonoids from different parts (stem, leaf, fruit and root) of the plant were introduced into the wells of the agar plates in different concentrations (0.5, 1, 1.5 and 2 mg/mL). A negative control well was also made with the extracting solvent DMSO. Positive control was made by the standard drug terbinafine. Plates were kept in sterile conditions for 30 min for pre-diffusion of extract to occur and then incubated at 27±2°C for 5 to 7 days.

The 'Inhibition Zone' (IZ) produced by the extracts was measured (mm) and the 'Activity Index' (AI) was calculated.

$$\text{Activity index} = \frac{\text{Inhibition zone produced by extrct}}{\text{Inhibition zone produced by standard}}$$

The experiment was performed in triplicate to minimize the chances of error and the mean values were calculated.

Determination of Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration

Extracts that showed significant inhibition and Activity Index (AI) in the agar well diffusion method against test pathogens, were further investigated for Minimum Inhibitory Concentration (MIC). Micro broth dilution method was performed to determine MIC values.[12]

Two-fold serial dilution of stock solution (10 mg/mL) of each extract was made and equal volume of broth was added to 96-well microtiter plates. To each well, 100 µL inoculum of standard size (1×10^7 cfu/mL) was added. Standard drug was used for positive control while fungal suspension was used for negative control. Microtiter plates were incubated at 27±2°C for 48 hr. After an incubation period, 10 mg/mL tetrazolium salt was prepared and 50 µL of this solution was added to each well and microtiter plate was incubated for 2 hr at 37°C. Pink colour appeared as a result of reaction of tetrazolium with active cells of fungi. The least concentration of extract that showed no colour change after incubation was considered as MIC of the extract. Minimum Fungicidal Concentration (MFC) was taken by subculturing 50 µL from each well showing no colour change reaction. Extracts with the least concentration showing no visible growth on subculturing were taken as MFC values.

Total activity

The Total Activity (TA) is a measure of the amount extracted from the plant in relation to MIC of the extracts/fraction/compound isolated. Total activity is the volume at which test extract can be diluted with the ability to kill dermatophytes. It is calculated by

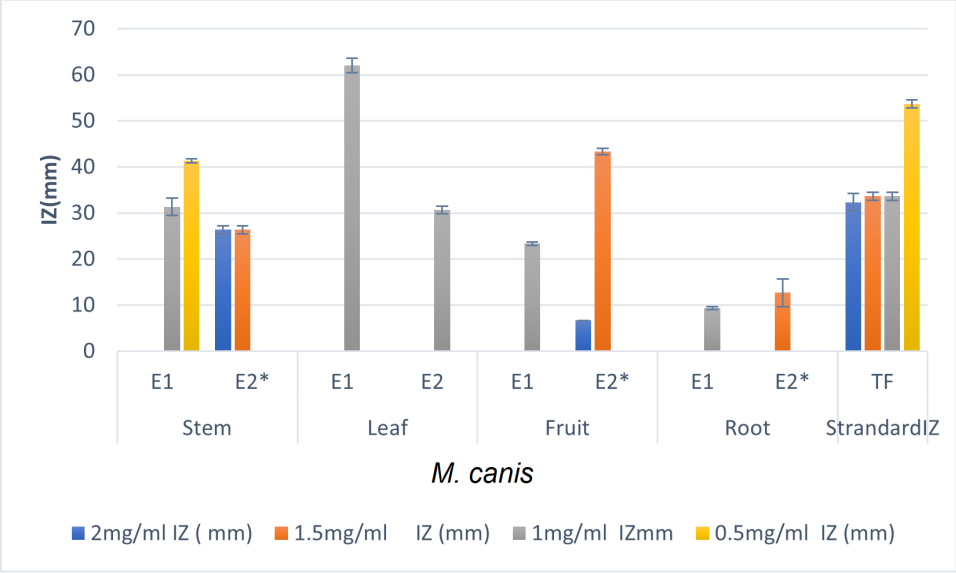

Figure 1: Antidermatophytic activity of flavonoids of *Euphorbia hirta* against *M. canis*. Inhibition zone (mm) of extracts from *Euphorbia hirta* against *M. canis*; Tested concentrations of extract against *M. canis* are 2 mg/mL, 1.5 mg/mL, 1 mg/mL, 0.5 mg/mL.

Table 1: Anti-dermatophytic activity of flavonoids of *Euphorbia hirta* against *Microsporum canis*.

Sl. No.	Plant part	Extract	*Microsporum canis*							
			2 mg/mL		1.5 mg/mL		1 mg/mL		0.5 mg/mL	
			IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)
1	Stem	E_1	41.33±0.409	0.783±0.013	39.33±1.855	1.166±0.017	(-)	(-)	(-)	(-)
		E_2	NG	NG	NG	NG	26.33± 0.881	0.78±0.023	26.33±0.881	0.816±0.017
2	Leaf	E_1	NG	NG	62±1.547	1.81±0.015	(-)	(-)	(-)	(-)
		E_2	NG	NG	30.66± 0.769	0.92±0.020	(-)	(-)	(-)	(-)
3	Fruit	E_1	NG	NG	23.33± 0.334	0.686±0.020	(-)	(-)	(-)	(-)
		E_2	NG	NG	NG	NG	43.33± 0.666	1.276±0.014	6.66± 0.879	0.203±0.020
4	Root	E_1	NG	NG	9.33± 0.333	0.276±0.029	(-)	(-)	(-)	(-)
		E_2	NG	NG	NG	NG	12.66± 2.999	0.37±0.034	(-)	(-)
5	Terbinafine	TF	53.66±0.888		33.66±0.881		33.66±0.881		32.33±1.855	

All values are in MEAN±SD (each plant extract tested in triplicate); E1: Free flavonoids, E2: Bound flavonoids, NG: No growth of dermatophyte (Absolute inhibition of test dermatophyte by the extracts), (-): No anti-dermatophytic activity of the extract at tested concentration, IZ: Inhibition Zone in mm (including 6 mm of well), AI: Activity Index, TF: Terbinafine.

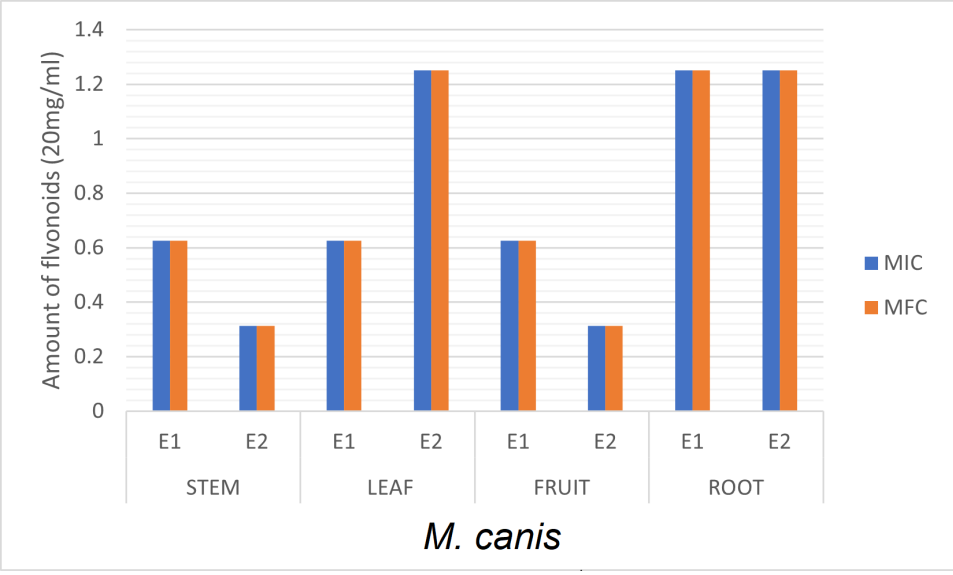

Figure 2: MIC and MFC of flavonoids from *Euphorbia hirta* against *M. canis*. MIC=Minimum inhibitory concentration; MFC=Minimum fungicidal concentration; E_1=free flavonoids; E_2=bound flavonoids.

dividing the amount of extract from 1 g plant material by the MIC of the sane extract or compound isolated and is expressed in mL/g.[13] In mathematical terms, it can be expressed as,

$$\text{Total Activity} = \frac{\text{Amount extracted from 1 gm plant material (mg|gdw)}}{\text{MIC of the extract (mg|mL)}}$$

RESULTS

Most of the flavonoid extracts of selected plants showed antidermatophytic potential against test dermatophytes i.e. *Microsporum canis, Trichophyton rubrum*, and *Epidermophyton floccosum*.

Antibiotic terbinafine was taken as standard drug for evaluation and comparison of bio efficacy of the extracts. Standard drug Terbinafine tested against *M. canis, T. rubrum* and *E. flocoosum* at different concentrations (0.5, 1, 1.5 and 2 mg/mL). The inhibition zone of Standard drug terbinafine i.e. recorded was 32.33 mm at 0.5 mg/mL; 33 mm at 1 mg/mL; 33.66 mm at 1.5 mg/mL; and 53.66 mm at 2 mg/mL against *Microsporium canis*. The inhibition zone of standard against *Trichophyton rubrum* was (49 mm at 0.5 mg/mL; 61.66 mm at 1 mg/mL; 73.33 mm at 1.5 mg/mL; and 73.66 mm at 2 mg/mL) whereas against *Epidermophyton floccosum* IZ recorded was 28.33 mm at 0.5 mg/mL; 33 mm at 1 mg/mL; 38.33 mm at 1.5 mg/mL; and 41.33 mm at 2 mg/mL. Twenty-four plant extract of flavonoids (free and bound) of *Euphorbia hirta* tested against *M. canis, T. rubrum* and *E. flocoosum* at different

Table 2: MIC and MFC of flavonoids of *Euphorbia hirta* against *Microsporum canis*.

Sl. No.	Plant part	Extract	*Microsporum canis*	
			MIC	MFC
1	Stem	E_1	0.625	0.625
		E_2	0.3125	0.3125
2	Leaf	E_1	0.625	0.625
		E_2	1.25	1.25
3	Fruit	E_1	0.625	0.625
		E_2	0.3125	0.3125
4	Root	E_1	1.25	1.25
		E_2	1.25	1.25

MIC: Minimum inhibitory concentration (mg/mL); MFC: Minimum fungicidal concentration (mg/mL); E1: Free flavonoid; E2: Bound flavonoid.

Table 3: Antidermatophytic activity of flavonoids of *Euphorbia hirta* against *Trichophyton rubrum*.

Sl. No.	Plant part	Extract	*Trichophyton rubrum*							
			2 mg/mL		1.5 mg/mL		1 mg/mL		0.5 mg/mL	
			IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)
1	Stem	E_1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		E_2	NG	NG	NG	NG	25.66±0.333	0.416±0.017	(-)	(-)
2	Leaf	E_1	NG	NG	26.33±1.452	0.356±0.029	(-)	(-)	(-)	(-)
		E_2	27± 1	0.366±0.023	22±1.452	0.313±0.029	(-)	(-)	(-)	(-)
3	Fruit	E_1	NG	NG	17.66±1.154	0.253±0.035	(-)	(-)	(-)	(-)
		E_2	62.33 ±1.201	0.836±0.088	45.33±1.763	0.623±0.024	(-)	(-)	(-)	(-)
4	Root	E_1	NG	NG	9.66±0.666	0.143±0.024	(-)	(-)	(-)	(-)
		E_2	NG	NG	31±1.527	0.423±0.026	(-)	(-)	(-)	(-)
5	Terbinafine	TF	73.66 ±0.333		73.33±1.154		61.66±1.20		49±0.57	

All values are in MEAN±SD (each plant extract tested in triplicate); E1: Free flavonoids, E2: Bound flavonoids, NG: No growth of dermatophyte (Absolute inhibition of test dermatophyte by the extracts), (-): No antidermatophytic activity of the extract at tested concentration, IZ: Inhibition Zone in mm (including 6 mm of well), AI: Activity Index, TF: Terbinafine.

Figure 3: Antidermatophytic activity of flavonoids of *Euphorbia hirta* against *T. rubrum*. Inhibition zone (mm) of extracts of *Euphorbia hirta* against *T. rubrum*; Tested concentrations of extract against *T. rubrum* are 2 mg/mL, 1.5 mg/mL, 1 mg/mL, 0.5 mg/mL.

concentrations (0.5, 1, 1.5 and 2 mg/mL) and their IZ, AI, MIC, MFC and TA were calculated as per given formula.

Microsporum canis

8/24 flavonoid extracts (free and bound) exhibited activity against *M. canis*, with most extracts showing resistance at higher concentrations (2 mg/mL). Free flavonoids (E1) from the stem, leaf, fruit and root demonstrated significant inhibition zones (IZ) against *M. canis*. The stem extract showed IZ at 2 mg/mL and 1.5 mg/mL, with IZ (IZ 41.33±0.409 mm, AI 0.783±0.013 mm) and (IZ 39.33±1.855 mm, AI 1.166±0.017 mm), respectively. Free flavonoids from leaf, fruit and root were active at a concentration of 1.5 mg/mL, with IZ (IZ 62±1.542 mm, AI 1.81±0.015 mm); (IZ 23.33±0.334 mm, AI 0.686±0.020 mm); and (IZ 9.33±0.333 mm, AI 0.276±0.023 mm), respectively. Bound flavonoids (E_2) from stem, fruit and root extracts exhibited IZ at 1 mg/mL and 0.5 mg/mL, while the leaf extract showed IZ at 1.5 mg/mL. The IZ values for bound flavonoids from stem and fruit, a were showed IZ at 1 and 0.5 mg /mL (IZ 26.33±0.881 mm, AI 0.78±0.023 mm; and IZ 26.33±0.881 mm, AI 0.816±0.017 mm); (IZ 43.33±0.666 mm, AI 1.276±0.014 mm; and IZ 6.666±0.879 mm, AI 0.203±0.020 mm)) but root showed their activity only at 0.5 mg /ml (IZ 12.66±2.999 mm, AI 0.37±0.034 mm), respectively. The bound flavonoid IZ for the leaf was recorded IZ 30.66±0.769 mm, AI 0.92±0.020 mm at 1.5 mg/mL (Table 1; Figure 3).

The MIC and MFC of free (E_1) and bound (E_2) flavonoids from stem, leaf and fruit were same (0.625 mg/mL and 0.625 mg/mL), while the free flavonoid root MIC and MFC 1.25 mg/mL were found. For bound flavonoids (E_2) from leaf and root, the MIC and MFC were 1.25 mg/mL. For stem and fruit, bound flavonoids had MIC and MFC of 0.3125 mg/mL (Table 2; Figure 4).

Total Activity (TA) of free flavonoids from stem, leaf, fruit and root was 80.928 mL/g, 26.24 mL/g, 20.752 mL/g and 2.376 mL/g, respectively. For bound flavonoids, the TA values were 100.48

Table 4: MIC and MFC of flavonoids of *Euphorbia hirta* against *Trichophyton rubrum*.

Sl. No.	Plant part	Extract	*Trichophyton rubrum*	
			MIC	MFC
1	Stem	E_1	(-)	(-)
		E_2	1.25	1.25
2	Leaf	E_1	1.25	1.25
		E_2	0.625	0.625
3	Fruit	E_1	1.25	1.25
		E_2	1.25	1.25
4	Root	E_1	1.25	1.25
		E_2	0.3125	0.3125

MIC: Minimum inhibitory concentration (mg/mL); MFC: Minimum fungicidal concentration (mg/mL); E1: Free flavonoid; E2: Bound flavonoid.

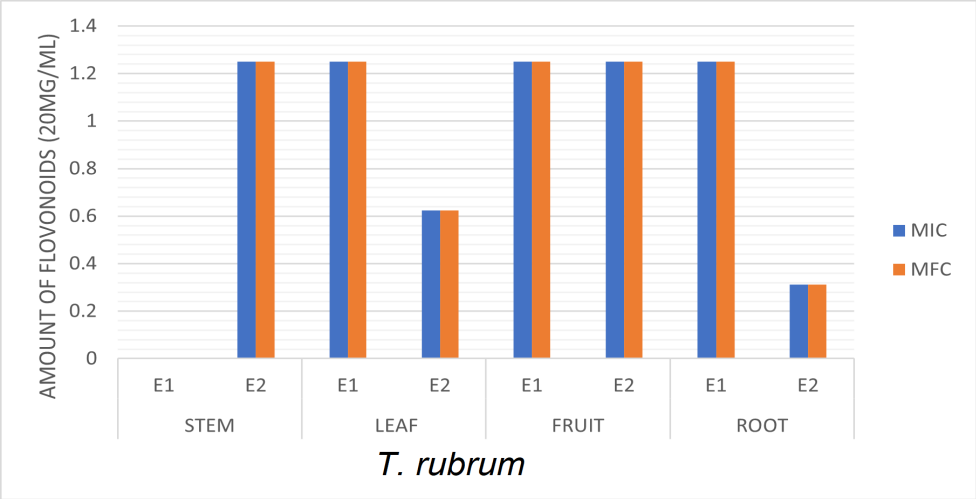

Figure 4: MIC and MFC of flavonoids from *Euphorbia hirta* against *T. rubrum*. MIC=Minimum inhibitory concentration; MFC=Minimum fungicidal concentration; E_1=free flavonoids; E_2=bound flavonoids.

Table 5: Antidermatophytic activity of flavonoids of *Euphorbia hirta* against *Epidermophyton floccosum*.

Sl. No.	Plant	Extract	*Epidermophyton floccosum*							
			2 mg/mL		1.5 mg/mL		1 mg/mL		0.5 mg/mL	
			IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)	IZ (mm)	AI (mm)
1	Stem	E_1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		E_2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
2	Leaf	E_1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		E_2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	Fruit	E_1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		E_2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	Root	E_1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		E_2	NG	NG	23.66±0.333	0.72±0.0152	(-)	(-)	(-)	(-)
5	Terbinafine	TR	41.33±1.20		38.33±0.881		33 ±1.527		28.33±0.881	

All values are in MEAN±SD (each plant extract tested in triplicate); E1: Free flavonoids, E2: Bound flavonoids, NG: No growth of dermatophyte (Absolute inhibition of test dermatophyte by the extracts), (-): No antidermatophytic activity of the extract at tested concentration, IZ: Inhibition Zone in mm (including 6 mm of well), AI: Activity Index, TF: Terbinafine.

Table 6: MIC and MFC of flavonoids of *Euphorbia hirta* against *Epidermophyton floccosum*.

Sl. No.	Plant part	Extract	*Epidermophyton floccosum*	
			MIC	MFC
1	Stem	E_1	(-)	(-)
		E_2	(-)	(-)
2	Leaf	E_1	(-)	(-)
		E_2	(-)	(-)
3	Fruit	E_1	(-)	(-)
		E_2	(-)	(-)
4	Root	E_1	(-)	(-)
		E_2	1.25	1.25

MIC: Minimum inhibitory concentration (mg/mL); MFC: Minimum fungicidal concentration (mg/mL); E1: free flavonoid; E2: bound flavonoid.

Table 7: Total activity of flavonoids of Euphorbia hirta extracts against *Microsporum canis, Trichophyton rubrum* and *Epidermophyton floccosum*.

Dermatophytes	Total activity (mL/gm)							
	Stem		Leaf		Fruit		Root	
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Microsporum canis	80.928	100.48	26.24	40.536	20.752	4.62	2.376	2.56
Trichophyton rubrum	(-)	24.88	13.12	81.072	20.725	1.16	2.376	10.26
Epidermophyton floccosm	(-)	(-)	(-)	(-)	(-)	(-)	(-)	2.56

E1: Free flavonoid; E2: Bound flavonoid; (-): Not active.Total activity (TA): Amount extracted from 1 g plant material (mg/g.d.w.)/MIC of the extract (mg/mL).

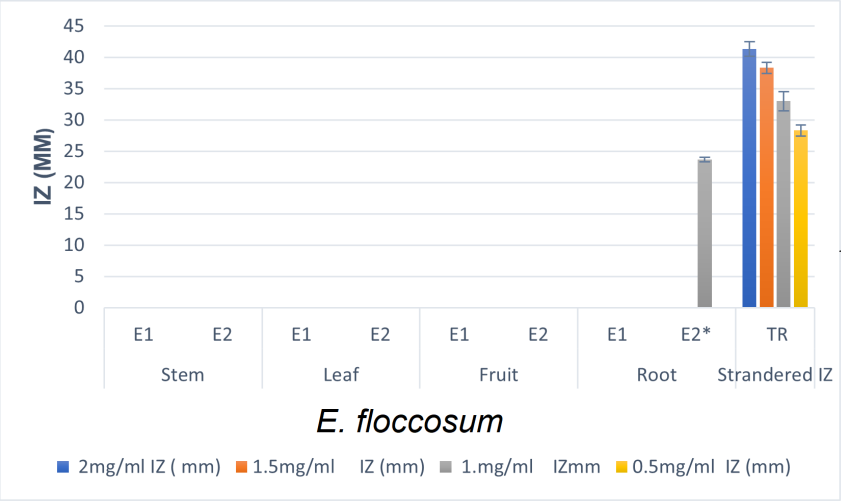

Figure 5: Antidermatophytic activity of flavonoids of *Euphorbia hirta* against *E. floccosum*. Amount of flavonoids (mg/gdw) extracted from *Euphorbia hirta*; Tested concentrations of extract against *E. floccosum* are 2 mg/mL, 1.5 mg/mL, 1 mg/mL, 0.5 mg/mL.

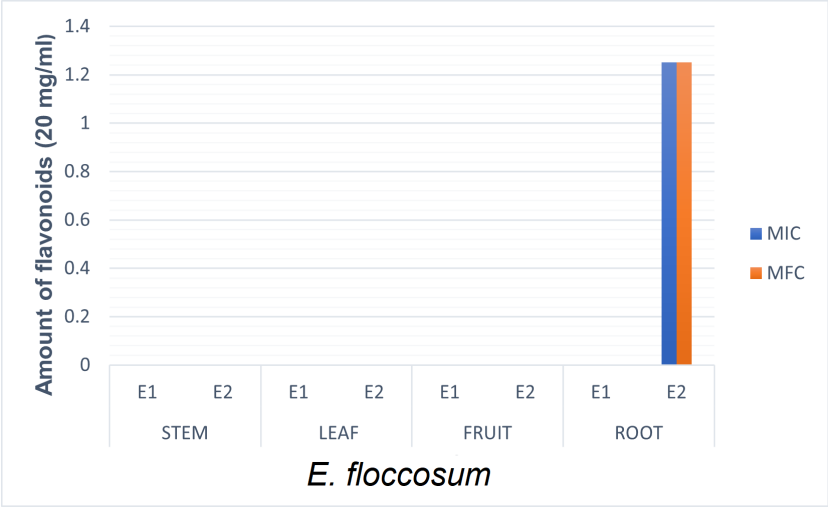

Figure 6: MIC and MFC of flavonoids from *Euphorbia hirta* against *E. floccosum*. MIC=Minimum inhibitory concentration; MFC=Minimum fungicidal concentration; E_1=free flavonoids; E_2=bound flavonoids.

mL/g, 40.536 mL/g, 4.62 mL/g and 2.56 mL/g, respectively (Table 7, Figure 5).

Trichophyton rubrum

8/24 flavonoid extracts (free and bound) exhibited antidermatophytic potential activity *T. rubrum*. The free flavonoids (E_1) from the stem were found to be potent against *T. rubrum* at various concentrations (0.5 mg/mL, 1 mg/mL, 1.5 mg/mL, 2 mg/mL). Free flavonoids (E_1) from leaf, fruit and root were active at 1.5 mg/mL. The Inhibition Zones (IZ) for leaf fruit and root extracts were found (IZ 26.33±1.452 mm, AI 0.356±0.021 mm); (IZ 17.66±1.154 mm, AI 0.253±0.035 mm) and (IZ 9.66±0.666 mm, AI 0.143±0.024 mm). Bound flavonoids (E_2) from the stem were active at 1 mg/mL, with IZ of 25.66±0.333 mm, AI 0.416±0.017 mm). Bound flavonoids (E_2) from the leaf and fruit were active at 2 mg/mL and 1.5 mg/mL, respectively. The IZ for bound flavonoids (E_2) from the leaf and fruit were recorded (IZ 27 ±1 mm, AI 0.366±0.023 mm and 22±1.452 mm, AI 0.313±0.029 mm); (IZ 62.33±1.201 mm, AI 0.836±0.088 mm and 45.33±1.763 mm, AI 0.623±0.024 mm) at 2 and 1.5 mg/mL. The bound flavonoids (E_2) from the root exhibited IZ of 31±1.527 mm, AI 0.423±0.026 mm at 1.5 mg/mL concentration (Table 3; Figure 6).

The MIC and MFC of free flavonoids (E_1) from the leaf, fruit and root were all found to be 1.25 mg/mL. For bound flavonoids (E_2), the MIC and MFC for stem and fruit were 1.25 mg/mL, while for leaf and root; they were 0.625 mg/mL and 0.3125 mg/mL, respectively (Table 4; Figure 7).

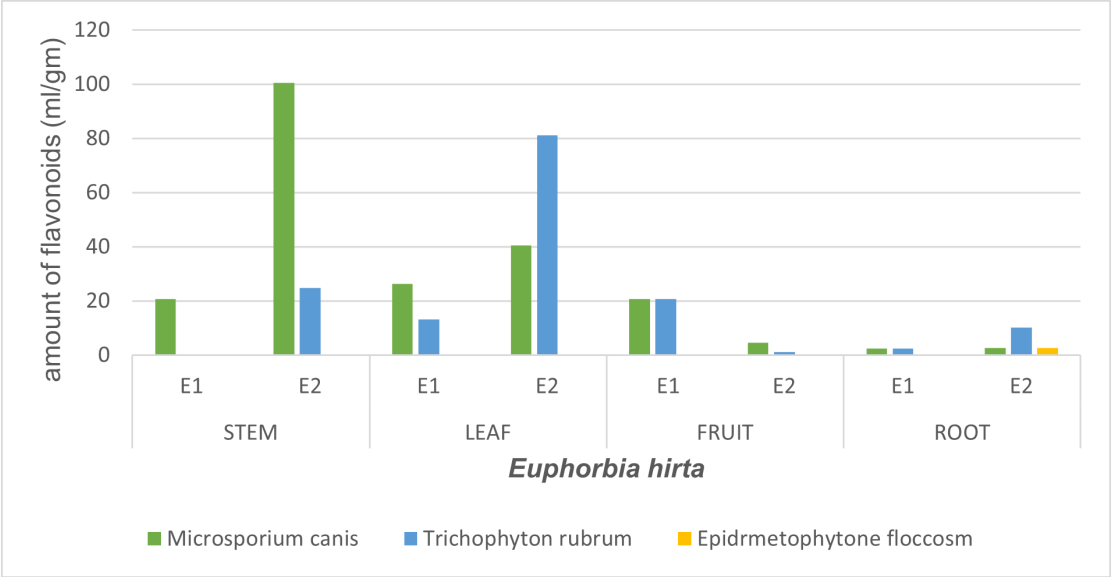

Figure 7: Total activity of flavonoids of *Euphorbia hirta* against *M. canis, T. rubrum, E. floccosum.*

Figure 8: *Euphorbia hirta*

Total activity (TA) of free flavonoids (E_1) from the leaf, fruit and root were recorded as 13.12 mL/g, 20.725 mL/g and 2.376 mL/g, respectively. For bound flavonoids (E_2), the TA values for the stem, leaf, fruit and root were 24.88 mL/g, 81.07 mL/g, 1.16 mL/g and 10.26 mL/g, respectively (Table 7; Figure 5).

Epidermophyton floccosum

Out of 24 extracts, four free flavonoid extracts (E_1) from the stem, leaf, fruit and root, as well as three bound flavonoid extracts (E_2) from the stem, leaf and fruit, exhibited no antidermatophytic activity against *E. floccosum*. However, one bound flavonoid extract (E_2) from the root showed IZ 23.66±0.333 mm, AI 0.72±0.015 mm at 1 mg/mL (Table 5; Figure 8).

The MIC and MFC of the bound flavonoid extract from the root were 1.25 mg/mL and Total Activity (TA) recorded was 2.56 mL/g (Tables 6 and 7; Figures 5 and 9).

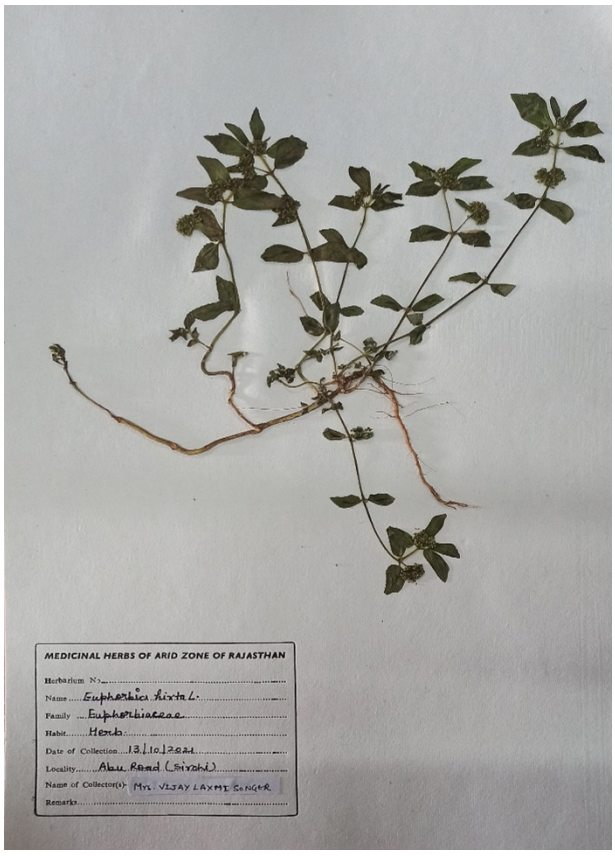

Figure 9: Herberium sheet of *Euphorbia hirta*

DISCUSSION

Most of the flavonoid extracts of selected plants showed antidermatophytic potential against test dermatophytes i.e. *Microsporum canis, Trichophyton rubrum*, and *Epidermophyton floccosum*. Antibiotic terbinafine was taken as standard drug for evaluation and comparison of bio efficacy of the extracts.

Three different concentrations (2, 1.5, 1 and 0.5 mg/mL) of both standard and extracts were taken for AI calculation. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were evaluated for plant extracts that showed antidermatophytic activity in the Agar well diffusion method. The total activity of the extracts was calculated by a well-established formula to relate the MIC of the extracts to the quantity extracted from 1 g of dried part of the plant.

A total of 24 extracts (free and bound flavonoids) from different parts of *Euphorbia hirta* were evaluated for their bio-efficacy against dermatophytes. Eight extracts out of 24 were not found active against *Trichophyton rubrum* and *Epidermophyton floccosum* at all tested concentrations (0.5 mg/mL, 1 mg/mL, 1.5 mg/mL and 2 mg/mL). Among 24 extracts, 11 showed 100% inhibition of *M. canis* and *T. rubrum* at 2 mg/mL concentration and no growth of fungi were observed in Petri plates in Agar Well assay. Four extracts showed same result at 1.5 mg/mL where no growth at all was recorded. Bound flavonoids from stem showed excellent potential against *M. canis* has no growth (i.e. 100% inhibition) was seen at 2 and 1.5 mg/mL concentration; whereas more or less similar IZ and AI were observed at 1 and 0.5 mg/mL. Remarkable IZ 62±1.547 mm and AI 1.841 were obtained for free flavonoids of leaf, while complete inhibition was seen at 2 mg/mL. All active flavonoid extracts were found to be fungicidal by exhibiting same values of MIC and MFC (Table 2; Figure 4). All extracts of free and bound flavonoids of selected plant showed excellent potential against *Trichophyton rubrum* and only one extract (free flavonoid of the stem) was found to be inactive. Fungicidal nature of all extracts against *T. rubrum* was seen with same values of MIC and MFC (Table 4, Figure 7). Bound flavonoids gave remarkable values of TA 81.072 mL/g against *T. rubrum* indicative of great antidermatophytic potential at even low concentrations (Table 7, Figure 5).

The most resistant test fungi in the present study were *Epidermophyton floccosum* against which most of the extracts were recorded inactive at tested concentrations. Only bound flavonoids from the root showed marginal IZ, AI, MIC and TA (IZ 26.66±0.333 mm, 0.7169; 1.25 mg/mL and 2.56 mL/g) (Tables 5-7; Figures 5, 8 and 9).

The present study evidently proves the antidermatophytic efficacy of a selected plant i.e. *Euphorbia hirta*. Metabolite profiling of the whole plant should be done especially for flavonoids that were found to be highly potent antidermatophytic compounds. Flavonoids from the plant could be used as the source for the development of alternative drugs for the topical treatment of dermatophytosis. Observations of the present investigation are indicative of the significant antidermatophytic efficacy of ethnobotanical medicinal plant *E. hirta.* It has been found to contain comparable antidermatophytic efficacy with that of standard drug terbinafine (most commonly used drug in the treatment of ringworm). Ethnobotanical surveys of medicinal plants for particular diseases should be coupled with metabolite profiling and scientific exploration of their hidden potentialities.

CONCLUSION

Euphorbia hirta is well known for its various remedial effects against several diseases such as diarrhea, dysentery, respiratory ailments, conjunctivitis and skin ailments. Decoction of dry plants is frequently utilized in the treatment of skin conditions.[14] (Kumar, *et al*., 2010).

The present study reveals the hidden antidermatophytic potency of flavonoids of the plant and suggests the identification and characterization of active principle/s and/or marker compounds present in the flavonoid extracts of the plant. Significant findings of the investigation also recommend pre-clinical studies of flavonoids of *E. hirta*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

IZ: Inhibition Zone; **AI:** Activity Index; **TA:** Total Activity; **MIC:** Minimum Inhibitory concentration; **MFC:** Minimum Fungicidal concentration; **DMSO:** Dimethyl sulfoxide; **MTCC:** Microbial type culture; **IMTEC:** Institution of microbial technology; **SDA:** Sabouraud dextrose agar; **CFU:** Colony forming unit; **E_1:** Ethyle ether; **E_2:** Ethyle acetate; **mg/mL:** Milligram per millilitre; **µL:** Microlitre; **mm:** Millimeter.

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